Tom5, Tom6 and Tom7. The receptors Tom20 and Tom70 are obviously only loosely associated as proved by Blue-native PAGE/Immuno blot studies [1]. Interestingly, there is evidence that the specific proteasome inhibitor lactacystin elevates Tom20 protein level [2]. Furthermore, this level might also depend on physiological demands [3]. To investigate possible variations in Tom20/TOM assembly on the single cell level, we searched for a method compatible with measurements in living cells. We employed fluorescence resonance energy transfer in combination with fluorescence lifetime imaging microscopy (FRET-FLIM) to investigate protein–protein interaction. Tom22 was fused to $eGFP_m$ as FRET donor and Tom20 to $DsRed_m$ or $DsRed_m$-Halo/TMR as FRET acceptor. Donor and acceptor-tagged Tom22 and Tom20 were coexpressed in mammalian HeLa cells. The average fluorescence lifetime t_{amp} was measured in presence and absence of acceptor. A mean FRET $(E=(1-tDA/tA)\times 100)$ of 3.49 ± 0.43 SD could be measured between Tom20-$eGFP_m$ and Tom22-$DsRed_m$. By using the double acceptor construct, the determined average FRET efficiency was significantly increased $(E=6.57\pm0.82$ SD). This is an almost twofold enhancement compared to using $DsRed_m$ as the only acceptor. Introduction of a 20 amino acid linker between Tom20 and the fluorescent protein tags decreased the FRET efficiency, indicating an unfavourable constitution for the acceptor function. For mitochondria treated with lactacystin, a specific inhibitor of the proteasome, we detected a significant rise in average FRET efficiency. We show that interaction between two candidate proteins of the TOM complex, Tom22 and Tom20, fused to $eGFP_m$ and $DsRed_m$-Halo, respectively, can be monitored in living cells using FRET-FLIM. Moreover, we found that specific inhibition of the proteasome apparently enhances the assembly of Tom20 into the TOM complex, resulting in increased FRET.

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18P8

The succinate oxidase supercomplex of the *Bacillus subtilis* aerobic respiratory chain

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The aerobic respiratory chain of the Gram positive bacterium *Bacillus subtilis* comprehends at least six proteins. Two main electron entry points, the alternative NADH:quinone oxidoreductase and the succinate:quinone oxidoreductase (SDH), reduce menaquinone. The reduced menaquinol can be oxidized by two distinct quinol: oxygen oxidoreductases, cytochromes *bd* and aa_3, or via the quinol: cytochrome *c* oxidoreductase b_1c_6/caa_3 cytochrome *c*:oxygen oxidoreductase pathway.

The membrane fraction of *B. subtilis* was investigated, after digitonin solubilization, by BN-PAGE *in-gel* activities, sucrose gradient and kinetics methods, to assess the presence of supercomplexes in the respiratory chain of this bacterium. Wild type and respiratory chain mutant strains were compared which allowed the identification of a new supercomplex composed of SDH and cytochromes b_1c_6 and caa_3.

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18P9

An integrated perspective of the *Escherichia coli* aerobic respiratory chain

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Respiratory chain supercomplexes have been reported in the past few years in both prokaryotes and eukaryotes, suggesting that these assemblies confer advantages to the oxidative phosphorylation processes, namely by enhancing the electron transfer efficiency and creating localized proton gradients.

The *Escherichia coli* aerobic respiratory chain is composed of at least six enzymes, namely type I and II NADH:quinone oxidoreductases, succinate:quinone oxidoreductase, cytochrome bo_3 oxygen reductase and type I and II cytochrome *bd* oxygen reductases, that accomplish the transfer of electrons from the reducing substrates NADH and $FADH_2$ to oxygen, with energy conservation. Recently, our group has identified, for the first time, three new supramolecular assemblies in the respiratory chain of this bacterium, a formate: oxygen oxidoreductase supercomplex containing the aerobic formate dehydrogenase (Fdo) and bo_3 and *bd*-I oxygen reductases, a supercomplex containing type I and II NADH:quinone oxidoreductases and a third supramolecular structure composed by succinate dehydrogenase and cytochrome *bd*-II [1,2]. This was achieved by BN-PAGE and *in-gel* activity detection as well as by sucrose gradient analyses of digitonin solubilized membranes from wild type and respiratory chain mutant strains and complemented by MALDI-TOF/TOF and LC-MS/MS analysis.

In the scope of these results, we've investigated the prevalence of such assemblies during the bacterial growth, complementing these data with the study of gene transcription and enzyme activities of the respiratory chain components. The obtained results were globally analyzed and correlations between gene transcription, enzyme activity and bacterial growth were established, providing a multilevel perspective of the *E. coli* respiratory chain.

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